BIOFILM FORMATION BY *Pseudomonas fluorescens*

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ABSTRACT

The formation of biofilms on heat exchange surfaces was studied using water contaminated with Pseudomonas fluorescens. The rate of deposition and the maximum amount of deposit decrease as the fluid velocity increases. The processes occurring at the interface and in the biofilm appear to govern the build-up of the deposit, together with the removal (shear stress) mechanism. Values of the attachment and biological growth rates were calculated from the changes observed in the biofilm thermal resistances after suppressing the addition of nutrients to the flowing water. In both cases there is a decrease for higher velocities.

INTRODUCTION

Biofouling - the accumulation of deposits on surfaces due to the adhesion and biological activity of microorganisms (or macroorganisms) - affects different types of industrial equipment, such as heat exchangers, cooling towers, filtration membranes and sensors. As a consequence, heat transfer efficiency is reduced, membranes are clogged, production losses are increased and corrosion may be induced on metal surfaces. In systems where cooling water is used (its temperature often reaching 30-40°C) biofouling can potentially occur. It may also occur in the process fluid side of heat exchange units, such as in the regenerative zone of milk pasteurizing plants, where thermoresistant micro-organisms can grow (1, 2).

BIOFOULING MECHANISMS

The main processes involved in biofilm formation in a flow system are (3): the formation of a "conditioning film" due to the adsorption on the deposition surface of organic macro-molecules (glycoproteins, polysaccharides, ...) contained in the fluid ("induction period"); the transport of microorganisms and dissolved components (which may act as nutrients) to the surfaces; the attachment of the microorganisms to the surface; the biological development of the deposit due to the growth and reproduction of organisms, as well as to the extracellular polymers and other products they excrete; the removal of parts of the biofilm caused by the hydrodynamic forces of the flowing fluid. This last process opposes the other four and, as a consequence, the build-up of biological deposits is often described by a sigmoidal curve leading to a final maximum amount of biofilm.

The quantitative aspects of biofilm formation are still poorly understood and few mathematical approaches are avaiable (4, 5), although a considerable amount of data has

already been published (5-8) reporting the effects of different variables on the extent of biofouling.

In this work, the formation of deposits caused by *Pseudomonas fluorescens* (often present in industrial cooling waters) was monitored by measuring heat transfer coefficients. The effects of fluid velocity and of the presence of nutrients were studied in an attempt to quantify some of the individual processes involved in biological fouling.

BIOFOULING MODEL

The basic structure of the model follows Kern and Seaton's concepts (9), who postulated that fouling is the result of a deposition process, occurring at a constant rate, and a simultaneous removal process, the rate of which increases with the thickness of the deposit. The amount of biofilm - here represented by its thermal resistance, R_f - will then increase according to the rate equation:

$$\frac{dR_f}{dt} = \phi_d - \phi_r \qquad (1)$$

where ϕ_d and ϕ_r are the deposition and removal fluxes, in terms of thermal units. Upon integration, Equation 1 leads to the asymptotic model (valid after the "induction" period):

$$R_f = R_f^{\infty} \, [1-\exp(-\beta.t)] \qquad (2)$$

where R_f^{∞} is the maximum value of R_f, and β is a parameter depending on the hydrodynamic conditions of the system and inversely proportional to the mechanical strength of the deposit. The following expressions are directly associated to this model:

$$\phi_d = \beta . R_f^{\infty} \qquad (3)$$

$$\phi_r = \beta . R_f \qquad (4)$$

In the case of biofouling, deposition involves two parallel phenomena:

1. Fluid and interface processes (ϕ_{d1}) - the transport of microorganisms to the deposition surface, where specific attachment mechanisms are established.

2. Fluid and biofilm processes (ϕ_{d2}) - the transport of nutrients to the deposit followed by the biological processes occuring in the biofilm (growth/reproduction, exopolymers production).

Since processes 1. and 2. are simultaneous, the overall deposition flux will be:

$$\phi_d = \phi_{d1} + \phi_{d2} \qquad (5)$$

ϕ_{d1} depends on the transport and attachment rates of bacteria and is constant with time (apart from the initial "induction" period).

ϕ_{d2} can also be assumed to be constant with time for the following reasons: a) the mass transfer rate of nutrients to the deposit does not change as long as the operating conditions are maintained; b) although the biofilm production rate could get higher on account of the increasing number of microorganisms attached, the resistance to the diffusion of nutrients throughout the deposit may lead to the establishment of a layer of active bacteria which represents a decreasing fraction of the total biofilm and compensaters the first effect.

MATERIALS AND METHODS

Tests were carried out in a rig containing a fermenter, a mixing vessel and two simulated heat exchangers (the test sections) - Figure 1.

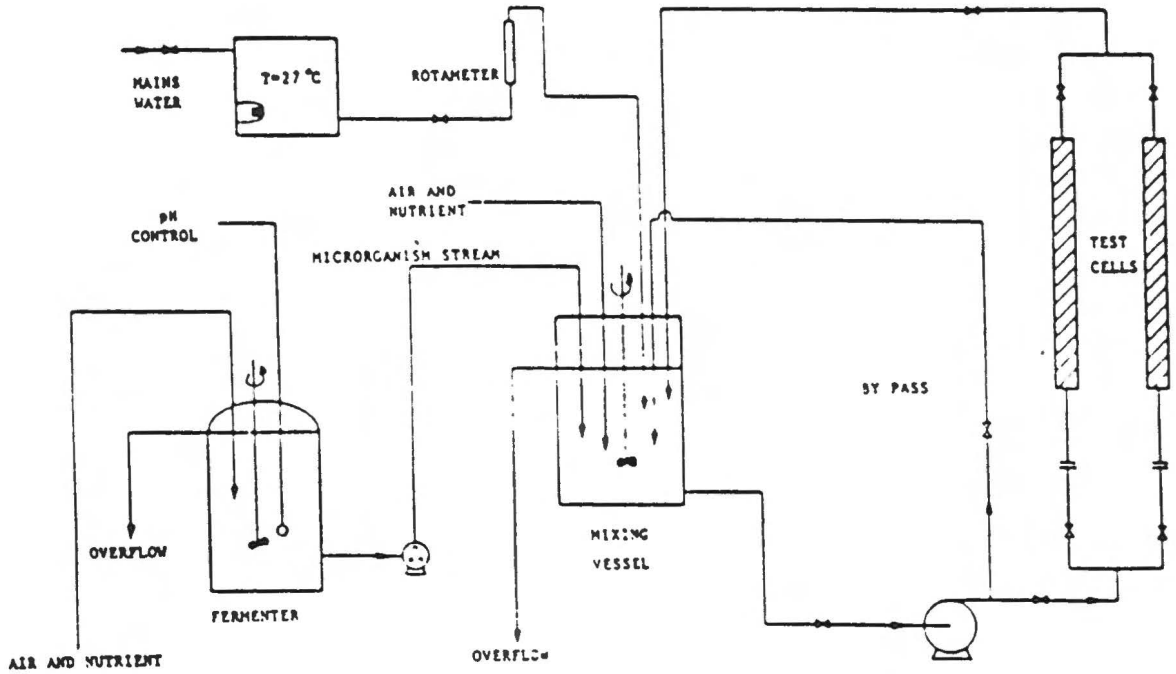

Figure 1 - Biofouling rig.

The fouling fluid was water at 27°C continuosly contaminated with the bacteria *Pseudomonas fluorescens* kept in a culture in the fermenter. A constant flow of nutrient (glucose, peptone and yeast extract) was added to the fermenter and to the mixing vessel, so that the concentration of glucose in the test cells was maintained at about 0.03 g/l. pH was controlled in the fermenter at the value of 7, no adjustments being needed in the mixing tank to maintain this value.

The test exchangers (Figure 2) were made of a perspex duct with a semi-circular cross section of 1.8 cm diameter, with an aluminium plate acting as the deposition surface. This metal plate was heated by water at 60°C circulating in an adjacent perspex duct with rectangular cross section. Temperatures were measured in four positions along the exchanger (A, B, C, D) using thermocouples placed in the fluid (T_3) and in the heat transfer wall (T_1 and T_2).

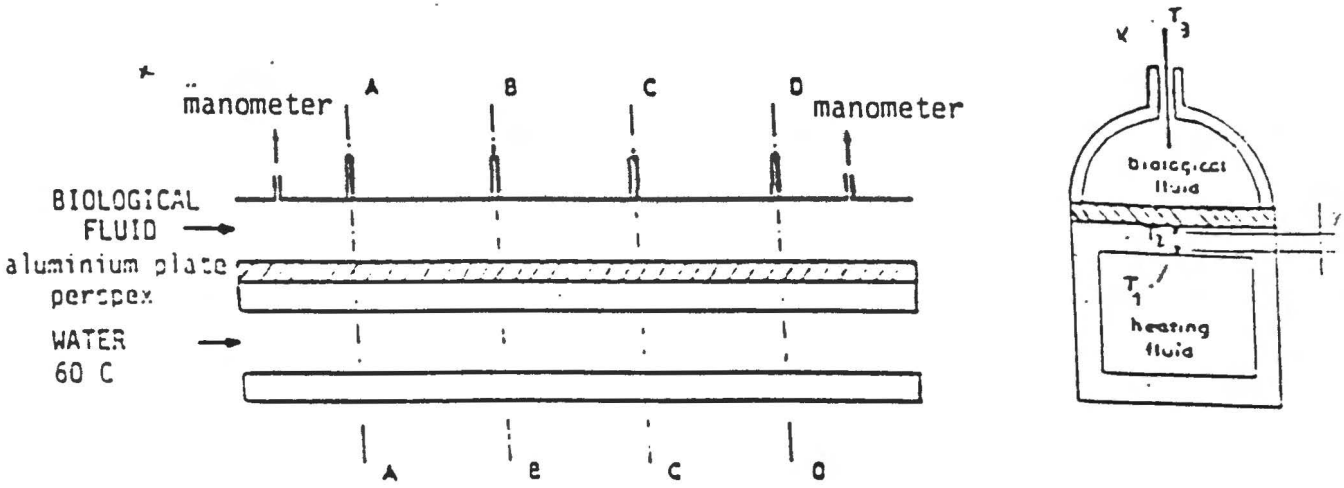

Figure 2 - Details of the test sections.

The overall heat transfer coefficient in each position was then calculated by

$$U = \frac{K_w}{y_w} \frac{T_1 - T_2}{T_1 - T_3} \qquad (6)$$

where y_w is the distance between thermocouples T_1 and T_2.

The effects of biofilm roughness on the convective heat transfer coefficient were determined from pressure drop measurements, as described elsewhere (10). The fouling resistances were evaluated as a function of time using the following equation:

$$R_f = \left(\frac{1}{U} - \frac{1}{U_o}\right) - \frac{1}{h_o}\left[\left(\frac{f_o}{f}\right)^p - 1\right] \qquad (7)$$

Several runs were performed at different fluid velocities corresponding to Reynolds numbers between 4200 and 12000. In three of them, the nutrient addition was stopped after the biofilm maximum thermal resistance was reached.

During the tests, the concentration of cells in the contamined water was determined by spreading a certain amount of fluid (after diluting it several times) in solid agar nutrient. After incubating for 24 hours at 27°C, the bacteria colonies were counted: the cell concentration was around 6×10^7 cells/ml in every run.

Total carbohydrates in the circulating fluid were analysed by the phenol-sulphuric colorimetric method (11), allowing the glucose concentration to be determined.

RESULTS AND DISCUSSION

The biofilms obtained in the tests showed an irregular topography, with average thickness reaching 1 mm (or even more) in the lower range of fluid velocities. A high density of biopolymeric material was observed on the scanning electron microscope (see photo at the end of this text).

Fouling curves

Figures 3 and 4 present typical fouling curves (R_f versus time) for position B in the test heat exchangers. Similar behaviour was found in the other measuring points. As expected, the asymptotic thermal resistances decrease with increasing Reynolds numbers, on account of the stronger removal action exerted by the fluid on the deposit. Furthermore, although the transport of bacteria to the surface is enhanced, their adhesion probability tends to decrease due to the greater shear stresses. If the prevailing mechanism in ϕ_{d1} is adhesion, then ϕ_{d1} will tend to decrease for higher velocities.

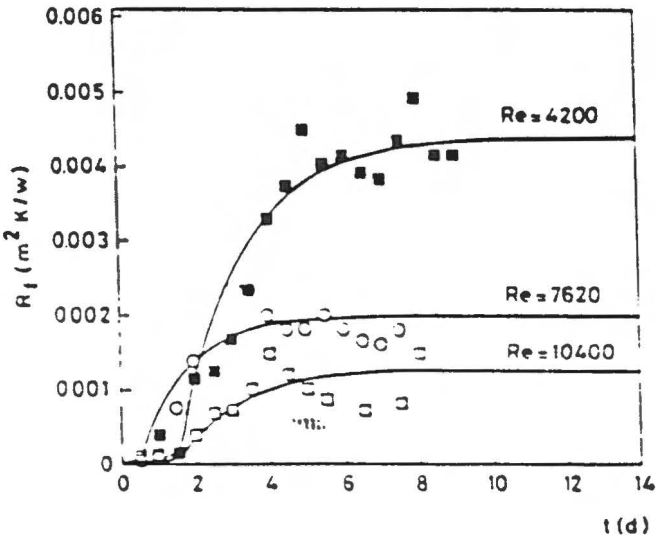

Figure 3 - Fouling curves.

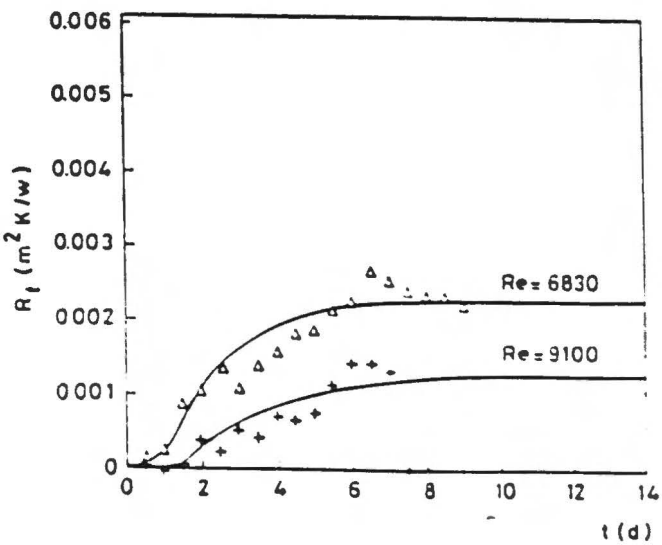

Figure 4 - Fouling curves.

Deposition flux (ϕ_d)

By fitting Equations 2 and 3 to the data of R_f versus time, values of ϕ_d were obtained for the different tests. The deposition flux (which includes, amongst others, the biological growth term) decreases continuously with increasing Reynolds numbers (Figure 5). This indicates that the transport mechanisms are not controlling the deposition process, since

mass transfer rates would increase with Re. Thus, as attachment and/or biological growth seem to be the limiting steps, an estimation of their contribution to the overall deposition flux was made. For that purpose, the study of the effect of nutrient suppression was found to be useful.

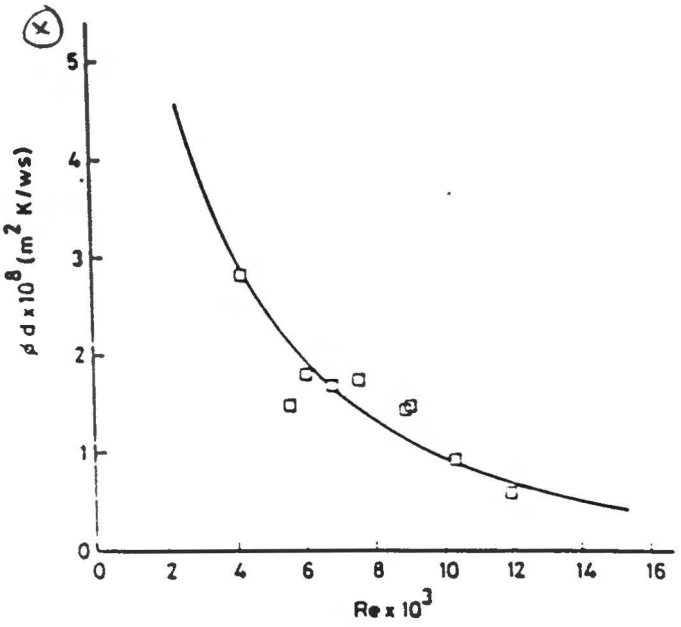

Figure 5 - Deposition flux versus Reynolds number.

Effect of nutrient suppression

Figures 6 to 8 show the reduction in the biofouling thermal resistance after removing the nutrients from the flowing water, for the cases of Re = 4200, Re = 6830 and Re = 8955 (fluid velocities: 0.34 m/s, 0.54 m/s, 0.71 m/s). The effect is more pronounced for high than for low Reynolds numbers, but in every case the fouling curve tends to a new (lower) asymptoptic resistance ($R^{\infty}_{f_{nr}}$) (Figures 6 to 8).

Taking $t = t_{nr}$ as the time when R_f started to decrease, the negative fouling rate for $t > t_{nr}$ can be described by an equation that does not contain the biological term, σ_{d2}, that is:

$$\left.\frac{dRf}{dt}\right|_{t > t_{nr}} = \sigma_{d1} - \sigma'_r \qquad (8)$$

where $\sigma'_r = \beta' R_f$ is the removal rate in the second phase of the experiment. σ'_r and β' are not necessarily equal to σ_r and β, since structural changes could have taken place in the inner layers of the deposit before the nutrients were removed. The integration of Equation 8 between the limits ($t = 0$, $R_f = R^{\infty}_{f_{nr}}$) and ($t$, R_f) results in:

$$R_f = R_{f_{nr}}^{\infty} \left[1 - \left(1 - \frac{R_f^{\infty}}{R_{f_{nr}}^{\infty}} \right) \exp\left(-\beta' t\right) \right] \tag{9}$$

and: $$\phi_{d1} = \beta' R_{f_{nr}}^{\infty} \tag{10}$$

After fitting Equation 9 to the experimental data for $t > t_{nr}$, values of ϕ_{d1} and ϕ_{d2} (Equation 5) were obtained and plotted in Figure 9.

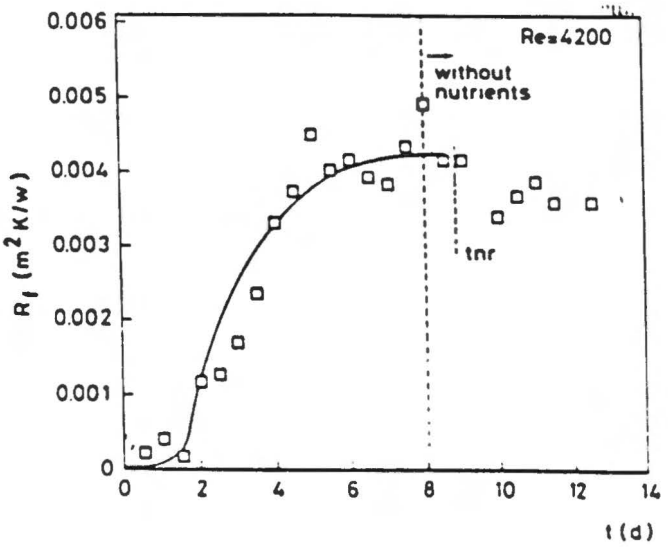

Figure 6 - Effect of nutrient suppression for Re = 4200.

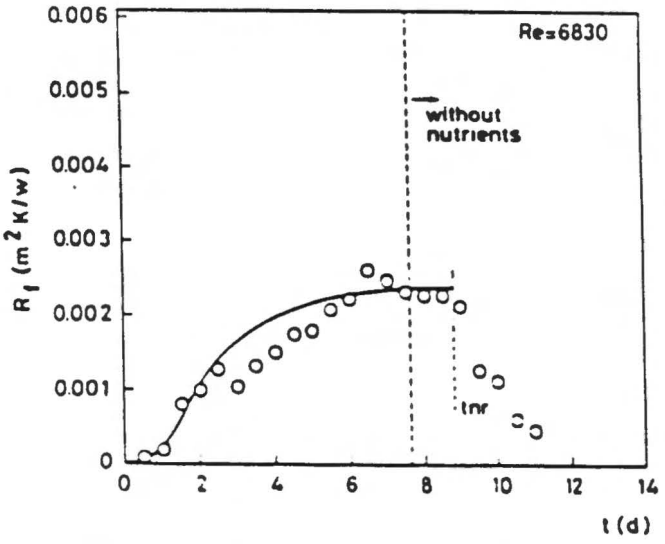

Figure 7 - Effect of nutrient suppression for Re = 6830.

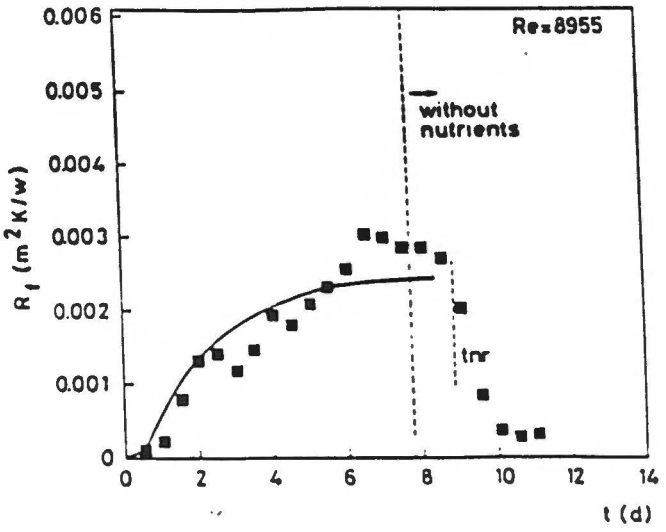

Figure 8 - Effect of nutrient suppression for Re = 8955.

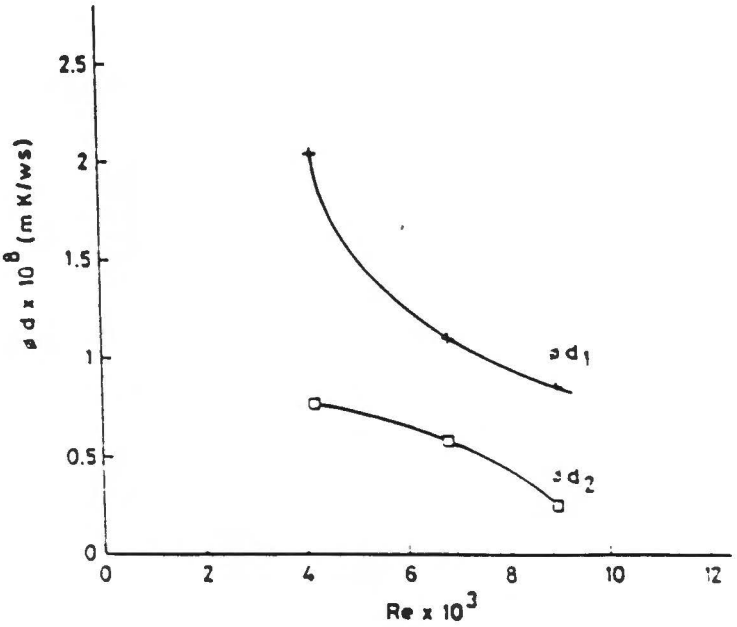

Figure 9 - ϕ_{d_1} and ϕ_{d_2} versus Reynolds number.

The relative importance of the fluid and interface processes (ϕ_{d_1}) is somewhat surprising. The mechanism of microbial attachement are rather complex since bacteria use pili and excreted polymers for this purpose (3), creating a deposit with an open structure filled with water. In the present case, due to the high nutrient content in the mixing tank it

is probable that the microorganisms produce biopolymers to a certain extent even before they reach the surface. This means that some of the "particles" that adhere to this surface are already complex structures, not simple bacteria. The thermal resistance added by such particles is thus higher than it would be expected from simple microorganisms, which explains the high values of ϕ_{d1}.

The deposition term related to the processes within the biofilm (ϕ_{d2}) also decreases with increasing Re. The compactness of the deposit tends to increase with fluid velocity, and this will make nutrient diffusion more difficult within the biofilm. These values are only partly in accordance with other published results (10) where ϕ_{d2} increased with fluid velocity for low Reynolds numbers. However, the previous assumption that ϕ_{d2} was much smaller (tending to zero) than ϕ_{d1}, in these range of Reynolds numbers, was not confirmed by the recent data obtained in the experiments where nutrient was suppressed.

Bott and Miller (8) also observed a pronounced reduction in biofilm weight after removing the nutrients for a fluid velocity of 0.5 m/s. A similar result was not found by these authors when the fluid velocity was 2 m/s, which means that ϕ_{d2} (biofilm production rate) did not play an important role in these flow conditions. Accordingly, the trend shown by the present data (Figure 9) suggest that for a velocity of 2 m/s (Re = 24780) the value of ϕ_{d2} would also be quite small.

There are also other aspects that have to be taken into account, such as the fraction of "active" layers in the biofilm. The present experiments do not allow to draw any conclusions about this question, which would also require the use of a more detailed biofouling model.

CONCLUSIONS

The results obtained in biofouling tests perfomed at different fluid velocities showed that the processes occurring at the interface (attachment) and in the biofilm (biological activity), together with the removal action of the hydrodynamic forces, determine the overall fouling rate.

Both types of processes are slowed down by high fluid velocities. In the case of attachment this tendency has been observed by several authors. Biological activity in the deposit could be expected to show a different behaviour, but the present data point out to the importance of compactness changes in the film that may increase the difficulty of nutrient diffusion to the inner layers of the deposit. The analysis of this question implies a more detailed model involving the concept of "active" and "inactive" layers in the biofilm.

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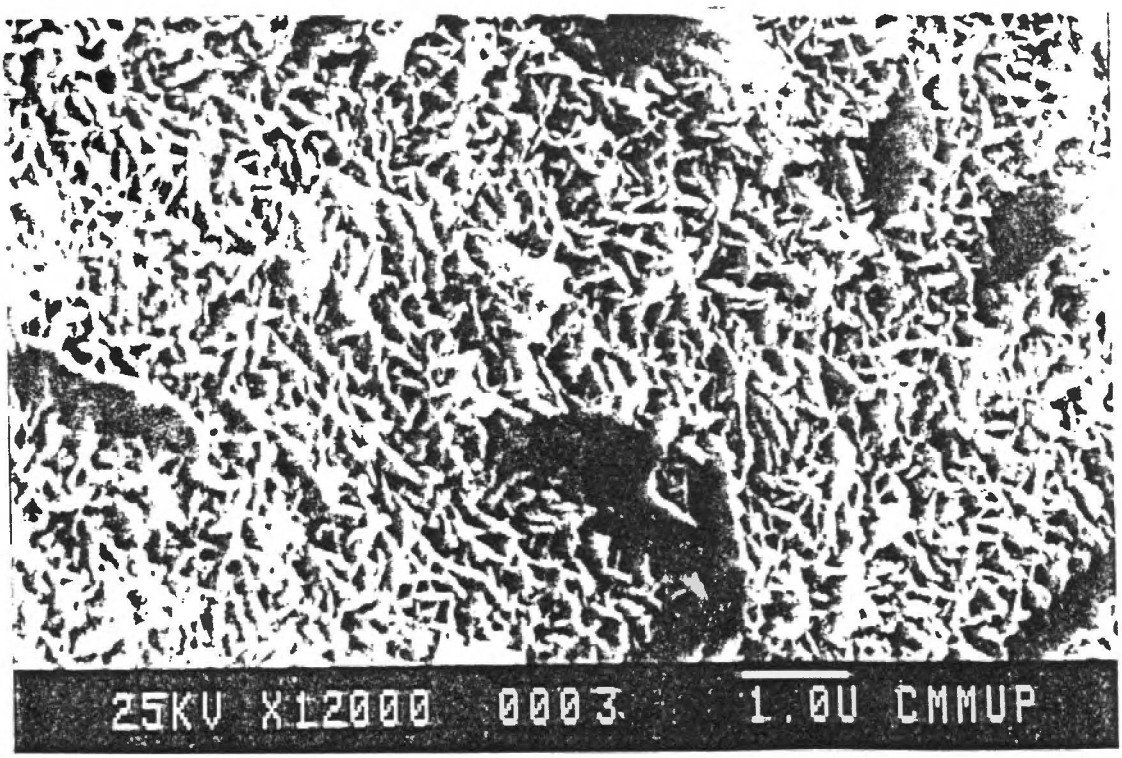

SEM picture of exopolymers in the biofilm

SYMBOLS

- f - friction factor with the fouled surface
- f_o - friction factor with the clean surface
- h_o - convective heat transfer coefficient (with the clean surface), W/m^2K
- K_w - thermal conductivity of the wall, W/m.k
- p = $0.68\ Pr^{0.215}$ (parameter)
- P_r - Prandtl number
- R_e - Reynolds number
- R_f - thermal resistance of the deposit, m^2 K/W
- R_f^{∞} - asymptotic thermal resistance of the deposit, m^2 K/W
- R_{fnr}^{∞} - asymptotic thermal resistance reached by the deposit after removal of nutrients, m^2 K/W
- t - time, s
- t_{nr} - time when R_f starts to decrease after suppression of nutrients, s
- T_1, T_2 - wall temperatures, K
- T_3 - fluid temperature, K
- U - overall heat transfer coeficient, $W/m^2.K$
- U_o - overall heat trasnfer coefficient with the clean surface, $W/m^2.K$
- y_w - distance between the thermocouples in the wall, m
- β - parameter related to the strenght of the deposit, s^{-1}
- β' - parameter related to the strenght of the deposit after removal of nutrients, s^{-1}
- α_d - deposition flux (overall), $m^2K/W.s$
- α_{d1} - deposition flux related to the transport and attachment of bacteria, $m^2K/W.s$
- α_{d2} - deposition flux related to the transport of nutrients and to the biological processes in the film, $m^2K/W.s$
- α_r - removal flux, $m^2K/W.s$
- α_r - removal flux after suppression of nutrients, $m^2K/W.s$

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